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Newsletter for September 2023

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Preliminary Discoveries in the Wnt Project

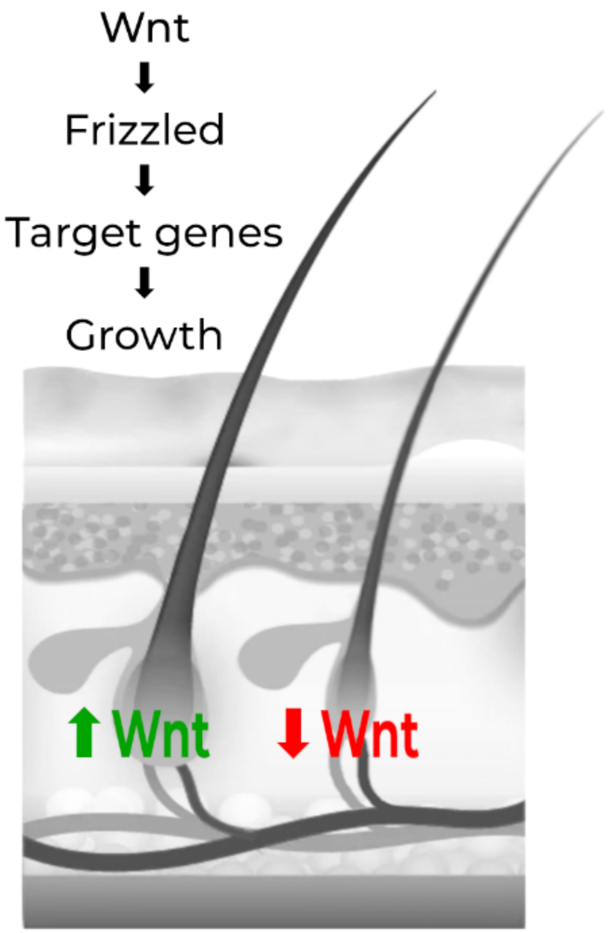
By Tilt Wolf, PhD cand.

We are excited to report that we have obtained preliminary positive results in our Wnt peptide project! In this project, we aim to find peptides that can modulate Wnt signaling in a receptor-specific way, because the Wnt pathway is an essential regulator of hair growth and morphology.

After much effort spent in finding experimental conditions where we could get positive control peptides to bind to Frizzled receptors - the targets of Wnt ligands - we found the secret to success. It was to use high-fidelity chemiluminescence instead of fluorescent detection, and to perform the experiment in Tris-buffered serum with a small amount of nonionic detergent.

Firstly, we found that one of our positive controls, Fz7-21 C10S - seen below at A3 in the spot grids - was able to bind Frizzled-1, reproducing known results in the literature. This tells us that our experiment conditions are working!

More excitingly, we see that our peptides, derived from human Wnt ligands based on known X-ray crystal structures, are able to bind to Frizzled-1. Some even bound more strongly than the positive control. To our knowledge, this is a brand new discovery, because no one has tried before to screen



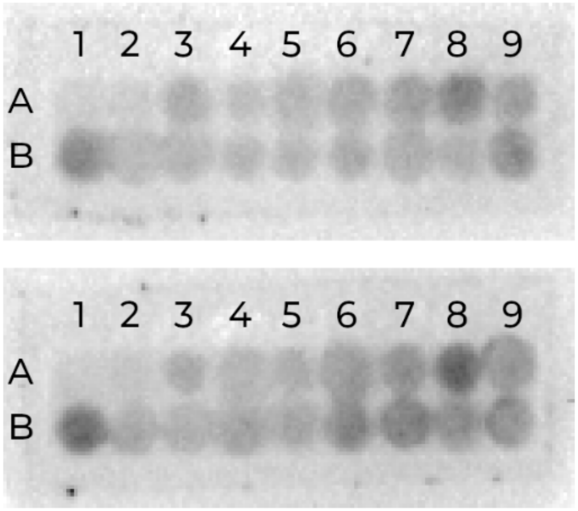


Figure 1 (top): Simplified summary, where high Wnt signaling in hair follicles causes strand thickening.

Figure 2 (bottom): Chemiluminescent images of spot peptide arrays treated with 2.5 µg/mL recombinant Frizzed-1; n=2 replicates.

synthetic fragments of Wnt ligands that have been O-palmitoylated - a difficult but necessary requirement for them to bind. O-palmitoylation is a natural process where palmitate, a fatty acid, is covalently joined to a serine residue in a protein.

We also noted an interesting trend - peptides that have lysine at position #14 can bind significantly more strongly to Frizzled-1 compared to peptides with arginine or glutamine (p=0.003, Mann-Whitney test). In fact, it was the only high confidence predictor of binding strength.

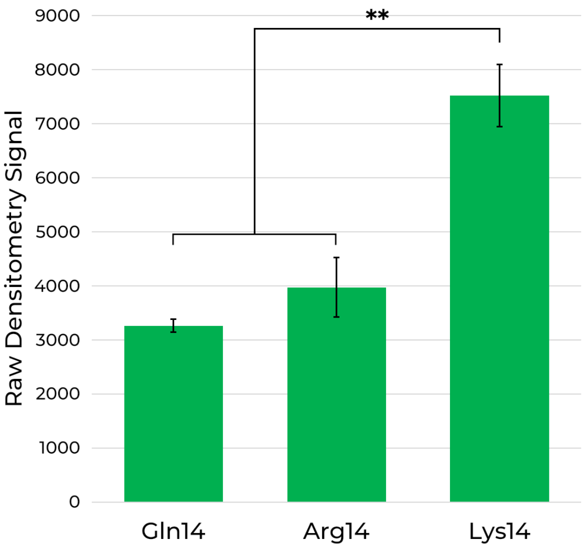


Figure 3: Higher spot peptide signals for residues with Lysine at position #14 compared to Arginine or Glutamine, p=0.003. Error bars are SEM values.

To better understand why Lys14 would be important for binding, I performed *in silico* modeling of the peptide-Frizzled-1 complex. The starting point was the known Wnt3-Frizzled-8 crystal structure (PDB:6AHY), which I substituted with Frizzled-1 and our strongest binding peptide from Wnt5a/b.

Normally, in Wnt proteins, the ends of the peptide are held together by a second disulfide bridge. However, our peptide only has one bridge, so the ends have more conformational freedom. In my modeling, it seems that this flexibility allows Lys14 (green) to bend downwards enough, allowing it to interact with Asp211 in Frizzled-1 to form a salt bridge.

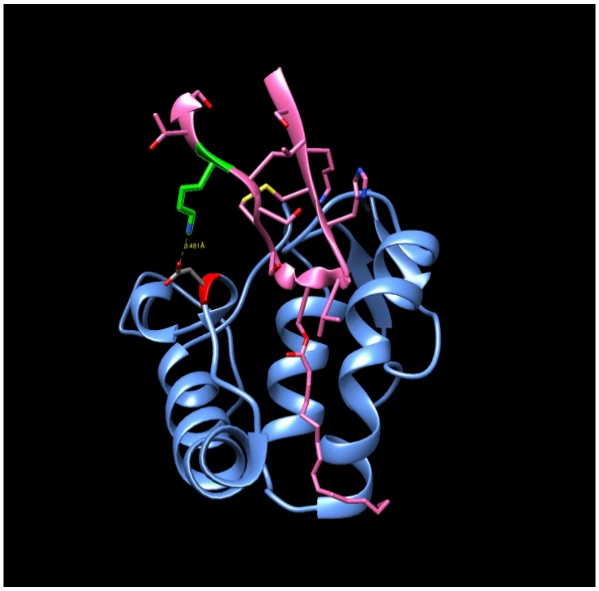


Figure 4: Computational modeling of Frizzled-1 (light blue) bound to one of our Lys14-containing peptides derived from Wnt5a/b (pink). Lys14 in the peptide is shown in green, and Asp211 in Frizzled-1 is shown in red.

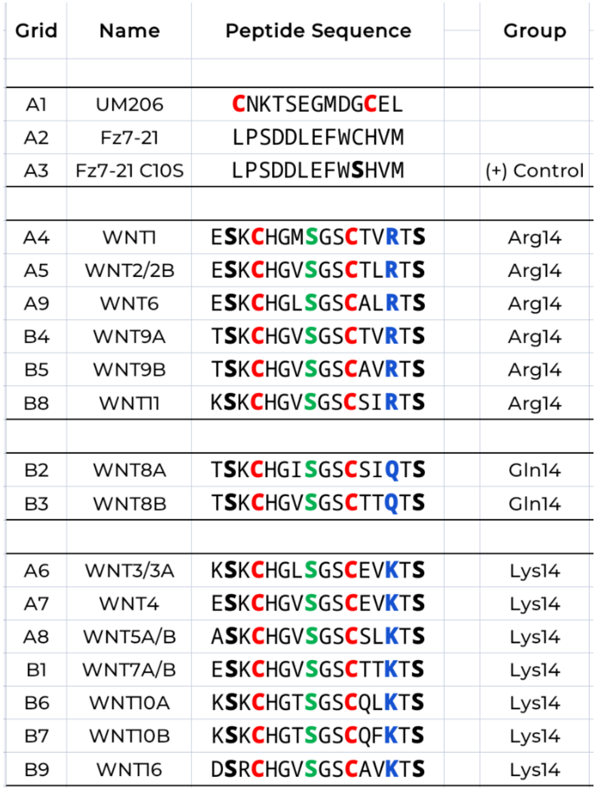
We hypothesize that the more concentrated positive charge of lysine is preferred for a salt bridge to form, compared to the more diffuse positive charge of arginine, which is distributed over more nitrogen atoms. Big thanks to Syralth for helping me to figure that one out!

While structural techniques like X-ray crystallography or NMR would be required for formal evidence of the binding mechanism, in practice, the impact of Lys14 on binding kinetics can be explored in the nearer term with evolutionary analysis and/or mutagenesis.

In the following weeks, we will test the remaining Frizzled receptors that are expressed in hair follicles, being Frizzled-5, -7, and -10. Our hope is that we will uncover preferential binding between different Wnt-derived peptides and different Frizzled receptors.

These peptides may serve as specific inhibitors, through competitive inhibition, of Wnt signaling. Since we already know that Wnt10b works to increase hair follicle size, we may be able to use these peptides to "turn down" follicle size, resulting in the finer hairs that make up fur (Lei et al., 2014).

Also, a big thank you to all of our supporters for helping make this research possible. These experiments require expensive reagents that we can only afford thanks to your support. Because of you, we can do the wet lab work needed to push us towards actual morphological freedom in real life.



Grid	Name	Peptide Sequence		Group
A1	UM206	CNKTSEGMDGCEL		
A2	Fz7-21	LPSDDLEFWCHVM		
A3	Fz7-21 C10S	LPSDDLEFWSHVM		(+) Control
A4	WNT1	ESKCHGMSGSCTVRTS		Arg14
A5	WNT2/2B	ESKCHGVSGSCTLRTS		Arg14
A9	WNT6	ESKCHGLSGSCALRTS		Arg14
B4	WNT9A	TSKCHGVSGSCTVRTS		Arg14
B5	WNT9B	TSKCHGVSGSCAVRTS		Arg14
B8	WNT11	KSKCHGVSGSCSIRTS		Arg14
B2	WNT8A	TSKCHGISGSCSIQTS		Gln14
B3	WNT8B	TSKCHGVSGSCTTQTS		Gln14
A6	WNT3/3A	KSKCHGLSGSCEVKTS		Lys14
A7	WNT4	ESKCHGVSGSCEVKTS		Lys14
A8	WNT5A/B	ASKCHGVSGSCSLKTS		Lys14
B1	WNT7A/B	ESKCHGVSGSCTTKTS		Lys14
B6	WNT10A	KSKCHGTSGSCQLKTS		Lys14
B7	WNT10B	KSKCHGTSGSCQFKTS		Lys14
B9	WNT16	DSRCHGVSGSCAVKTS		Lys14

Figure 5: List of tested Wnt-derived peptides grouped by residue at position #14.

Gender resources project: HRT content expansion

By Zennith, PhD

Late in 2022, we unveiled the first version of our Resources for Gender Affirmation, focusing on what people seeking affirmation of their gender can do today. We had realized that, despite the wide array of resources available online, it's difficult for a reader just starting out on a gender affirmation journey to find accessible, authoritative resources in one place.

We've been actively working on doing what we can for gender affirmation since then as well!

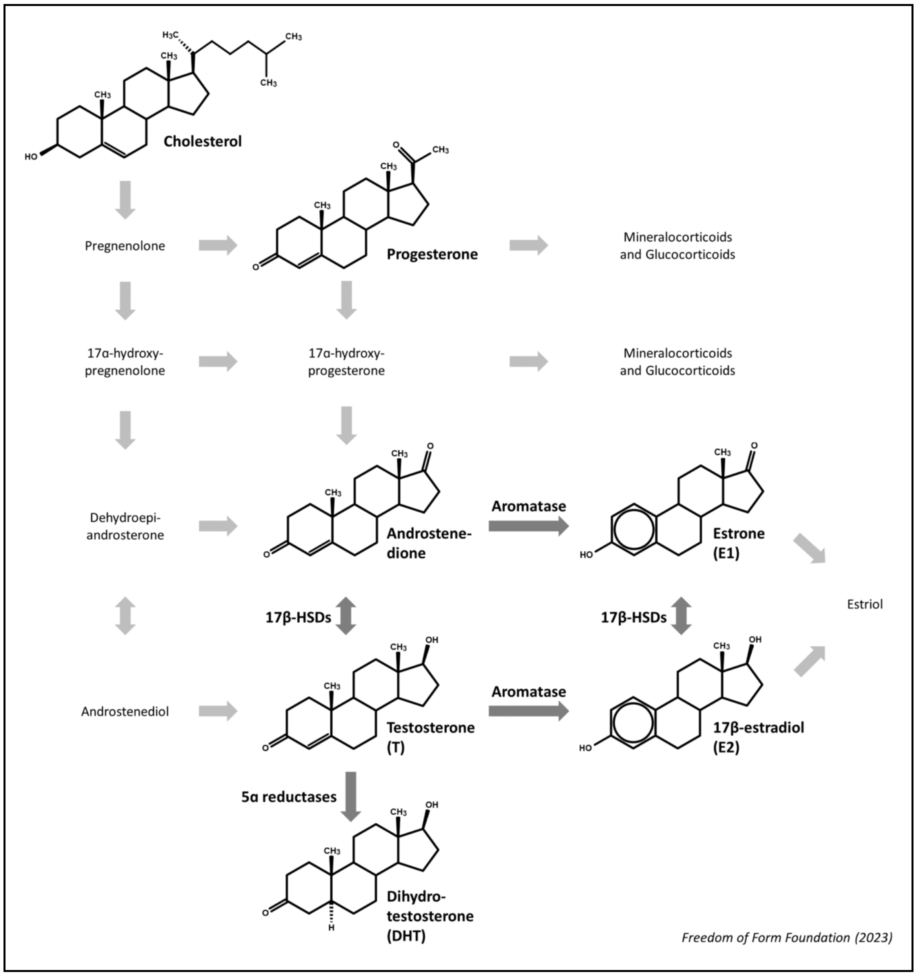
In May of this year, Roo and Elapids hosted a live Q&A session for members of our Discord community. They covered… a lot. They touched on medical, social, and legal transition; standards of care; their personal experiences with transitioning; voice transitioning; and even fashion advice! There were a lot of questions and the Q&A session took the entire 3 (3!) hour timeblock we suspected was overkill. It's extremely fulfilling for us to be able to help people like this, and we can't wait for the next one!

We want to help equip people with an overview of the information they need to be an active participant in their medical care, and Hormone Replacement Therapy (HRT) is a big part of that.

We initially covered what the expected effects of HRT, whether feminizing or masculinizing, are for a person's body. We are now working on expanding this to cover how HRT works, and even getting into specific information about medications and routes of administration (pills, injections, skin patches, etc).

We've just taken the wraps off of a new page, Biological Concepts in HRT! Here, we aim to help the reader understand how sex hormones work, and why HRT can affect so many different systems across the body.

The terms and concepts we introduce, while "optional" or "bonus points" for readers, may help you get more comfortable talking with medical professionals about specific choices in medications, and understand why some things would work better than others in particular situations.



Hormones including progestogens (including progesterone), androgens (including testosterone), and estrogens (including estradiol) are closely related to each other in biochemistry terms. Notable enzymes including 5α reductase and aromatase are shown.

We introduce the most relevant hormone names: progesterone, a couple different androgens including testosterone and DHT, and estrogens including estrone and estradiol. You'll probably come across the terms "5α reductase" and "aromatase" as well, and we briefly introduce what those enzymes do.

We then introduce concepts of how the body's cells interpret sex hormones and react to them, and connect these concepts to how HRT drugs actually work. Finally, we mention why it can matter whether a medicine is taken as a pill, injection, or skin patch, in terms of a medicine's effectiveness and side effects.

We try to stay only at high-level concepts here. Figuratively speaking, we're trying to show you how an overall forest looks, and if you're interested in looking at individual trees, now you'll know what to look for!

Again, this is "bonus points" reading and you should mainly rely on advice from medical professionals. We hope it's interesting and helps you become a more active participant in your healthcare!

In the coming weeks, we will further expand our HRT content to cover specific medicines, routes of administration, effects, and side-effects. While the specific medicine information will stand independently, it may make more intuitive sense when read together with Biological Concepts in HRT!

Thanks so much for reading and we hope this helps!